

PCTWORLD INTELLECTUAL
PROPERTY ORGANIZATION
Intern

INTERNATIONAL APPLICATION PUBLISHED

WO 9602248A1

(51) International Patent Classification⁶ :A61K 31/38, 31/34, C07D 333/54,
333/56, 307/79, 307/80

A1

(43) International Publication Date: 1 February 1996 (01.02.96)

(21) International Application Number: PCT/US95/08455

(22) International Filing Date: 6 July 1995 (06.07.95)

(30) Priority Data:
08/275,895 15 July 1994 (15.07.94) US(71) Applicant: ELI LILLY AND COMPANY [US/US]; Lilly
Corporate Center, Indianapolis, IN 46285 (US).(72) Inventors: FAHEY, Kennan, Joseph; 5047 Bird Branch Drive,
Indianapolis, IN 46268 (US). KOPPEL, Gary, Allen; 7823
Sunset Lane, Indianapolis, IN 46260 (US). CULLINAN,
George, Joseph; Rural Route 1, Box 468, Trafalgar, IN
46181 (US).(74) Agents: LAMMERT, Steven, R. et al.; Barnes & Thornburg,
1313 Merchants Bank Building, 11 South Meridian Street,
Indianapolis, IN 46204 (US).(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH,
CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE,
KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN,
MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE,
CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT,
SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML,
MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ,
UG).**Published***With international search report.*

(54) Title: CANNABINOID RECEPTOR ANTAGONISTS

(57) Abstract

This application discloses that certain arylbenzo[b]thiophene and benzo[b]furan compounds block or inhibit cannabinoid receptors in mammals. It also discloses novel compounds which are antagonists of the cannabinoid receptors, and pharmaceutical formulations which contain the compounds.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

-1-

CANNABINOID RECEPTOR ANTAGONISTS

This invention relates to aryl-benzo[b]thiophene and benzo[b]furan compounds which are active antagonists of the CB-1 (cannabinol-1) receptor in the mammalian central nervous system.

Cannabinoids are compounds derived from the *cannabis sativa* plant which is commonly known as marijuana. The most active chemical compound of the naturally occurring cannabinoids is tetrahydrocannabinol (THC), particularly (-)- Δ^9 -THC. This compound was isolated and identified in the 1960's and since that time there has been an ever increasing scientific investigation of the effects and pharmacology of the cannabinoids. However, prior to discovery of THC, the effects and pharmacology of marijuana use have been known for several thousand years. Both the uses and abuses of marijuana are recorded from the earliest human records. Marijuana based medicants have been known for centuries and have been a mainstay of many folk, herbal remedies.

Among the many beneficial pharmacological properties attributed to marijuana are: analgesia, lowering blood and intra-ocular pressure, and anti-emetic activity in both mammals and man. Indeed, currently, there is much debate over whether marijuana use should be legalized in certain cases, such as its use in cancer patients for ameliorating the nausea induced by chemotherapy or to lower intra-ocular pressure in glaucoma patients. After the elucidation of THC, several synthetic compounds were discovered and have been used clinically for the treatment of cancer patients, among these are: Nabilone, Nabortate and Levonantrodol. However, although these drugs are useful, they have to a greater or lesser extent some of the negative pharmacologic properties of THC and thus, are limited in their general use.

As marijuana's beneficial effects have been long known, so to its negative effects have been well documented. Notable in the negative pharmacology associated with marijuana (and later shown to be associated with THC) are:

-2-

psychological distortions of perception, loss of short-term memory, loss of motor coordination, sedation, and euphoria. Long term use of marijuana is considered by many to lead to addiction. Throughout the long history of marijuana its use and abuse have been intertwined.

5 Until the 1980's, the mechanism by which the cannabinols, most specifically THC, acted on the central nervous system was obscure. With the advent of very potent, radiolabelled, synthetic THC agonists (CP 55,940, HU210, and
10 HU211), the search for the molecular basis of THC pharmacology began to be elucidated.

 In 1988, it was determined that there was a specific receptor which bound Δ^9 THC as well as the other synthetic agonists. Using radio autography, the cannabinol receptor
15 was found to be localized in the hippocampus area of the rat brain(see: Herkenham, M., Ann. NY Acad. Sci., **645**: p. 19-32 (1992) and references therein). (For a review of this chronology, see: Mechoulam, R., et al., CNS Drugs, **2**(4), p. 255-260 (1994)). Subsequently, it was discovered that there
20 was another, distinct receptor which appears to be primarily located in the peripheral tissues, especially in the immune system (see: Lynn, A.B. and Herkenham, M., J. Pharm. and Exp. Ther., **268**(3), p. 1612-1623 (1994)). Both receptors have
25 been purified, amino acid sequenced, cloned, and expressed in experimental cell lines. The two receptors which bind both the cannabinoids and their synthetic agonists have been designated as: CB-1, the receptor located in the central nervous system, and CB-2, the receptor found in peripheral
30 tissues. It is generally agreed that much of the cannabinoid pharmacology, associated with its central nervous system effects and which is most germane to this invention, is directly related to the action of the CB-1 receptor. Synthetic and natural compounds which are agonists of the CB-1 receptor, demonstrate the expected experimental and human
35 pharmacology, while closely related compounds which bind poorly to CB-1 do not. (For a review of these findings, see:

-3-

Mechoulam, R., et al., *Biochem. Pharm.*, **48**(8), p. 1537-1544 (1994)).

5 In 1992, it was discovered that the endogenous ligand for the CB-1 receptor is anandamide, N-ethanolamine amide of arachidonic acid (see: Devane, W.A., et al., *Science*, **258**, p. 1946-1949. (1992)). The discovery of this new neuro-transmitter has initiated an intense investigation into the regulation and pharmacology of anandamide. Preliminary results indicate that animals treated with exogenous
10 anandamide demonstrate behavior similar to those treated with cannabinoids. Because of the parallelism of cannabinoid and anandamide pharmacology and the area of the location in the brain of the CB-1 receptor, there is a growing body of evidence that anandamide is a key regulator of functions such
15 as sensory perception, cognition, memory, pain perception, and mood modulation. Since it is very clear in humans what the agonism of the CB-1 receptor does with cannabinoids, it would seem reasonable by the same extrapolation to predict the likely pharmacology of antagonists of the CB-1 receptor
20 would possess.

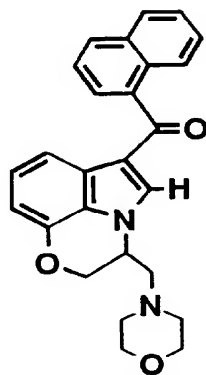
Therefore, in patients suffering from loss of sensory perception, cognition, and mood changes such as lethargy and depression, conditions which are often associated with the use of marijuana, there is a strong implication that a
25 controlling factor exacerbating these events would be an inappropriately high or unregulated control of anandamide - CB-1 interaction. An anandamide - CB-1 antagonist would be useful in conditions where patients exhibit these symptoms.

There are two reports in the art of cannabinol receptor
30 partial agonists or antagonists which are not either anandamide or cannabinoid analogs. The first of these compounds, an aminoalkylindole, a partial agonist, (see Fig.1) is revealed in Pacheco, M., et al., *J. Pharmacol. Exp. Ther.*, **257**, p. 170-183 (1991) and Compton, D.R., et al., *J. Pharmacol. Exp. Ther.*, **263**, p. 1118-1126 (1992). The
35 antagonist, a halo-aryl pyrazole (SR 141716A) (see Fig.2), was revealed in a patent application EP 0576357A1. The CB-1

-4-

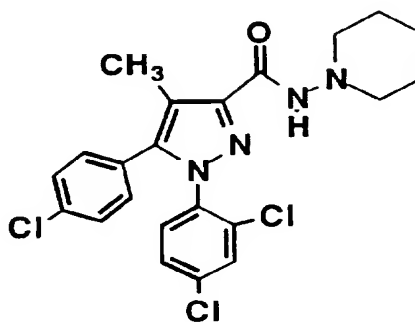
antagonist, SR141716A, has been shown to block the actions of both cannabinoids and anandamide in *in vivo* and *in vitro* models (see: Rinaldi-Carmona, M., et al., FEBS Letters, 350, p. 240-244 (1994)).

5



WIN-55,212

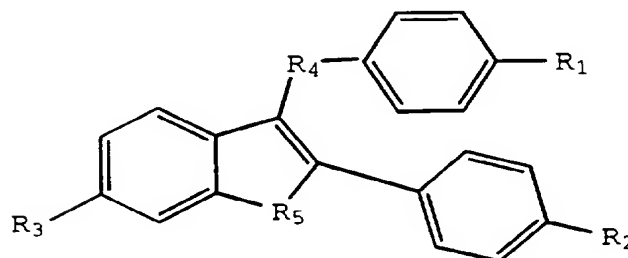
Fig. 1



SR 141716A

Fig. 2

This invention provides a compound of formula



I

10

in which

R¹ is C₁-C₄ alkoxy, trifluoromethylsulfonyloxy, hydroxy or cyano;

15 R² and R³ are each independently C₁-C₄ alkyl or C₁-C₄ alkoxy;

R⁴ is CO, CHOH or CH₂; and

R⁵ is O or S, provided that when R¹ is hydroxy, R⁵ is O, for use in a method of antagonizing one or more of the
20 actions of anandamide at cannabiniol-1 receptors in a mammal.

-5-

Certain compounds of formula I are believed to be novel and are provided as another aspect of the invention. Accordingly, the present invention provides a compound of formula I wherein

5 R^1 is cyano;
 R^2 and R^3 are each individually C₁-C₄ alkyl or C₁-C₄ alkoxy;

R^4 is carbonyl; and R^5 is oxygen or sulfur.

10 The invention also provides pharmaceutical formulations which include a novel compound of formula (I) as active ingredient in combination with a pharmaceutically acceptable carrier, diluent or excipient.

15 The term "C₁-C₄ alkyl" represents a straight or branched alkyl chain having from one to four carbon atoms. Typical straight or branched C₁-C₄ alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and t-butyl.

20 The term "C₁-C₄ alkoxy" represents an oxygen atom connected directly to the C₁-C₄ alkyl group to form an ether moiety. Examples of C₁-C₄ alkoxy groups include methoxy, ethoxy, propoxy and butoxy.

R^1 is preferably methoxy, trifluoromethylsulfonyloxy, hydroxy or cyano. Most preferably R^1 is cyano.

25 R^2 is preferably methyl or methoxy.

R^3 is preferably methoxy.

R^4 is preferably carbonyl.

R^5 is preferably -O-.

30 Particularly preferred compounds are those in which R^1 is cyano, R^2 is methoxy, R^3 is methoxy, R^4 is carbonyl and R^5 is O; or R^2 is methyl, R^3 is methoxy, R^4 is carbonyl and R^5 is O; or R^2 is methoxy, R^3 is methoxy, R^4 is carbonyl and R^5 is S.

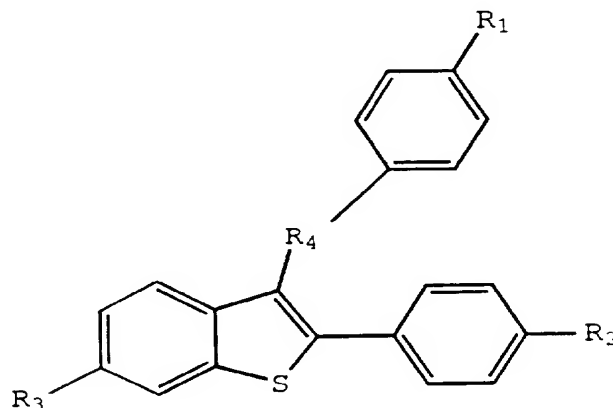
35 The compounds of formula I belong to two related chemical groups, the benzothiophene group and the benzofuran group.

-6-

1. Benzothiophene Group

This group of compounds includes all of the compounds where R⁵ is S, shown below:

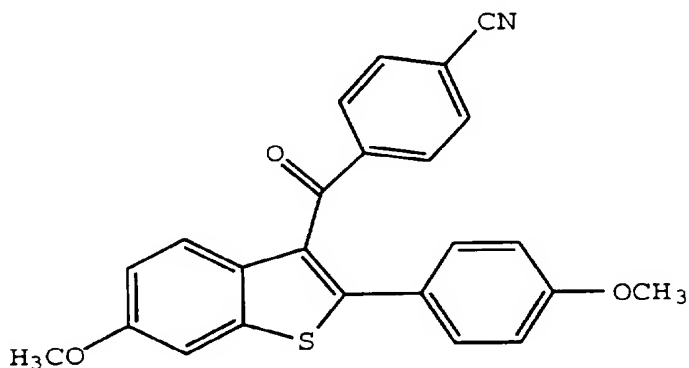
5



10

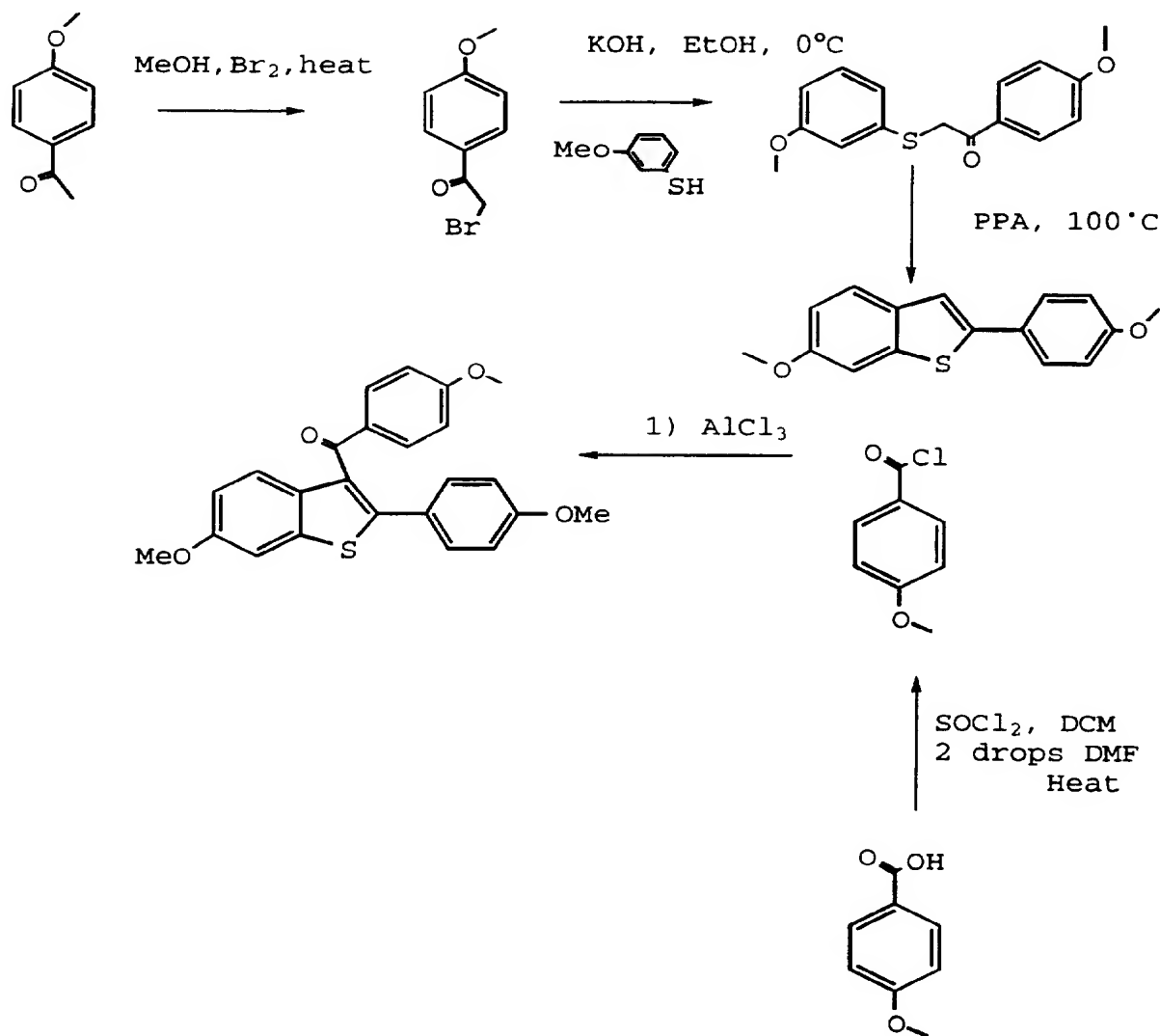
wherein R¹-R⁴ are as defined above. Of this group of preferred compounds, the most preferred compound has the R¹ group is CN; methoxy groups at R² and R³; and R⁴ is carbonyl. This substitution defines the most preferred compound, [6-methoxy-2-(4-methoxyphenyl)benzo(b)thien-3-yl][4-cyanophenyl]methanone which has the following structure:

15



The general scheme for synthesizing the preferred benzothiophene compounds is shown as Scheme I:

-7-

Scheme I

5

Benzothiophene synthesis, such as in the scheme shown above, is also well documented in the art. Jones, et al., J. Med. Chem., 1984, Vol. 27, No. 8, pp. 1057-1066, disclose the basic scheme shown above, while variations of this scheme are found in U.S. Patents 4,133,814; 4,358,593; 4,380,635; and 4,418,068.

10

-8-

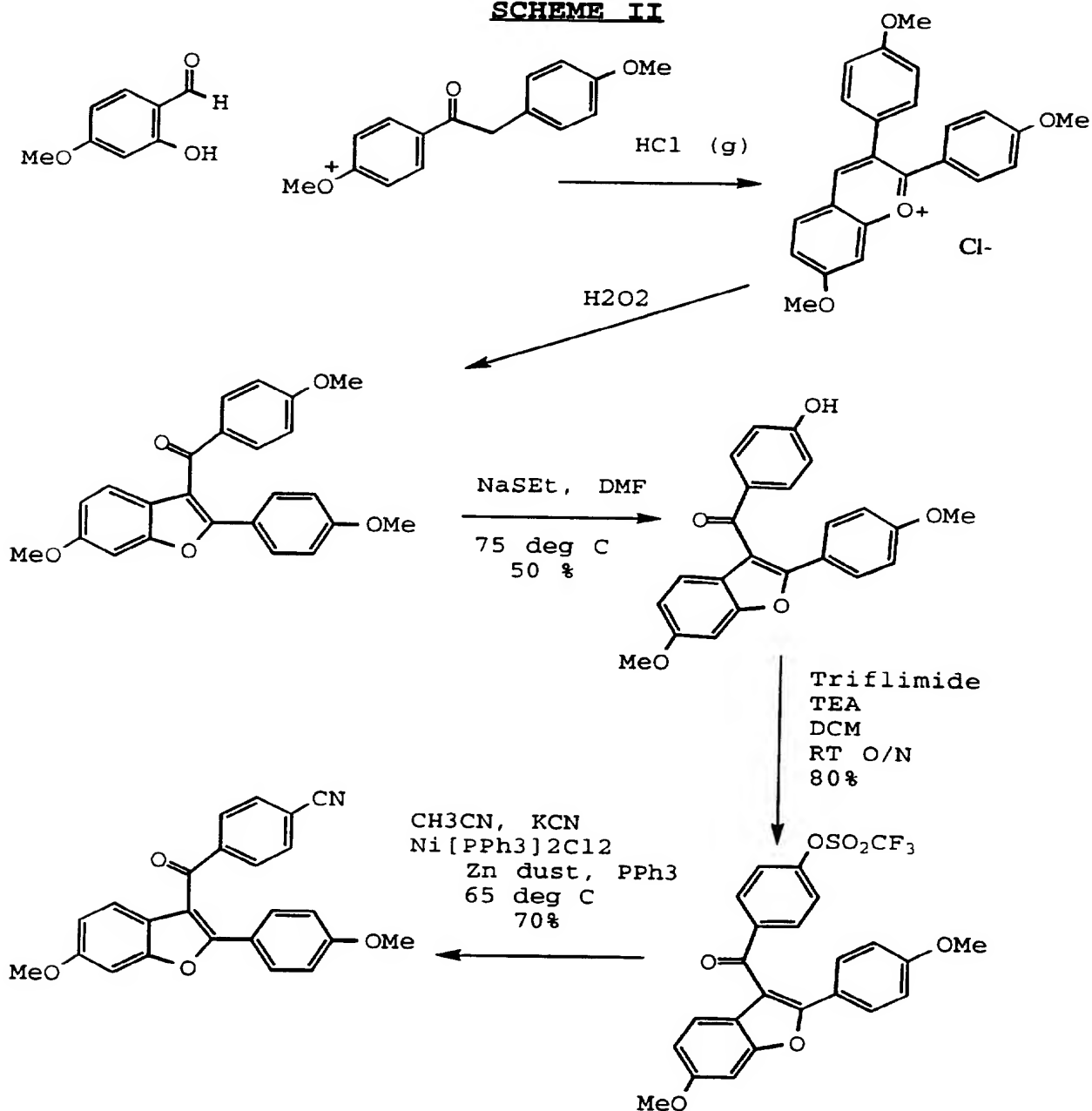
As shown in the above Scheme I the starting material of 4-methoxyacetophenone was brominated in methanol to form the halo intermediate shown. An aryl sulfide and base is reacted with this intermediate to form the diaryl intermediate linked by the sulfur - acetyl bridge. Cyclization of this intermediate to the desired benzo(b) thiophene backbone is achieved by reacting with polyphosphoric acid at elevated temperatures. As shown, the 4-methoxyphenyl moiety tends to arrange via cationic rearrangement to the 2-position of the benzothiophene.

The 2-aryl-benzo(b) thiophene intermediate is then reacted with an aryl acyl halide under Friedel-Crafts conditions. The electrophilic substitution takes place primarily on the three position (C₃) of the thiophene ring as shown. To produce the most preferred compound of this group, the 4-methoxy group is substituted by a cyano or nitrile group, which requires the presence of more catalyst due to low electrophility.

20 2. Benzo(b)furan Group

The general schemes for synthesizing compounds of this group is shown below as Scheme II:

-9-

SCHEME II

The above scheme generally depicts the synthesis of the most preferred compound of the benzofuran group, [6-methoxy-2-(4-methoxyphenyl)benzo[b]furan-3-yl]-3-(4-cyanophenyl)-methanone. Synthesis of the 3-(4-methoxyphenyl) intermediate compound is described in detail by Durani and Kapil, A

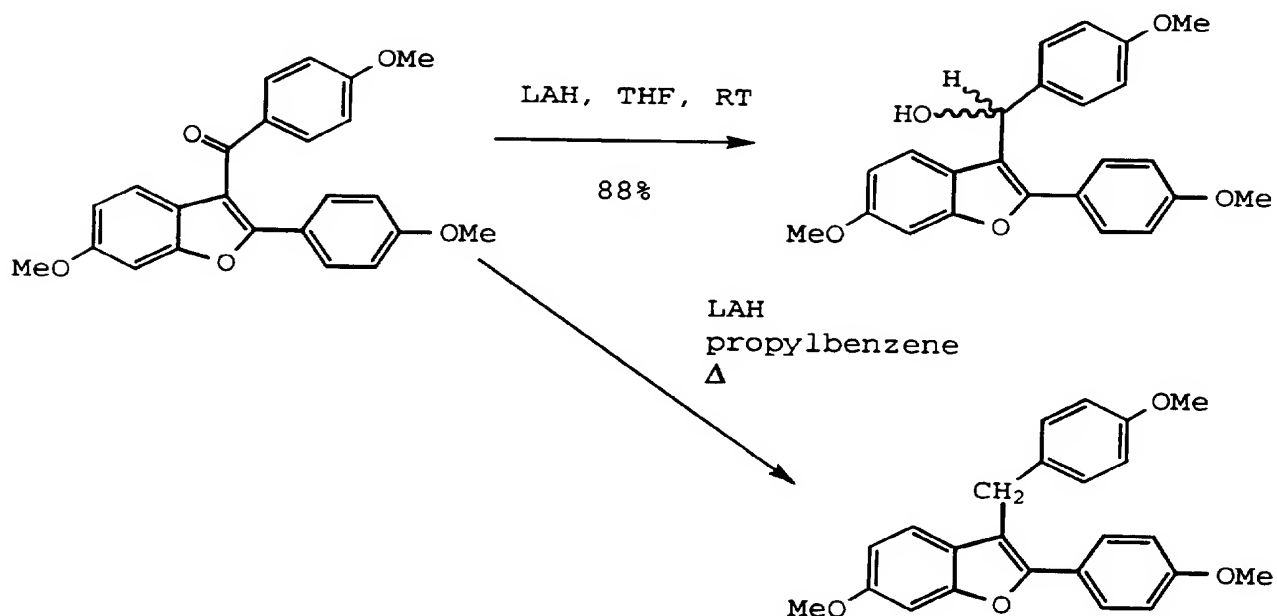
-10-

convenient Synthesis of 2-Aryl-3 Aroylbenzo[b] furans, Indian Journal of Chemistry, Vol. 22B, May 1983, pp. 489-490. As shown in Scheme II, 2-hydroxy-4-methoxy benzaldehyde is reacted with a diaryl ketone in a strong acid to form the benzopyrilium salt intermediate compound shown. This intermediate is then oxidized to produce the [6-methoxy-2-(4-methoxyphenyl)-benzo[b]furan-3-yl]-3-(4-methoxyphenyl)methanone intermediate which is well-known in the art.

This intermediate is then dealkylated by a well-known procedure to form the 4-hydroxy intermediate shown. A two-step process is then employed to convert the 4-hydroxy moiety to a nitrile group as shown above. First the hydroxy group is converted to a triflate ester derivative and then this compound is reacted with a cyanide source in the presence of a catalyst to complete the conversion to the preferred compound. The general procedure for converting a methoxy moiety to a cyano moiety is shown in part in Chambers and Widdowson, Nickel Catalysed Conversion of Phenol Triflates to Aromatic Nitriles and Acids, J. Chem. Soc. Perkin Trans. I, 1989, p. 1365. Preferred reagents for the conversion are triflimide ((CF₃SO₂)₂NPhenyl) and triethylamine in a non-polar solvent to effect the hydroxy-triflate conversion; acetonitrile, potassium cyanide, and Nickel-bis-triphenylphosphine chloride with zinc dust metal and triphenyl phosphine to effect the triflate to cyano conversion. As with scheme I, derivative compounds of the preferred compound are easily prepared by one skilled in the art or by the same chemistry.

Other benzo[b] furan derivatives are depicted in Scheme III shown below:

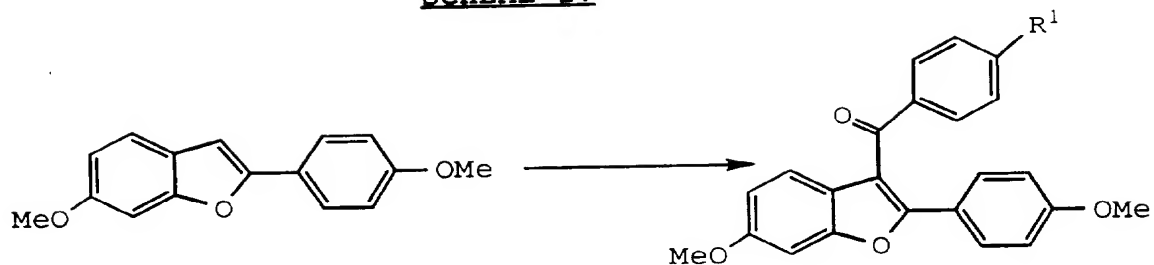
-11-

SCHEME III

The above scheme depicts the synthesis of various benzo[b]furan derivatives with different bridges between the furan ring and the 3-(4-methoxyphenyl)ring. As shown in Schemes I and II, the typical bridge is a carbonyl group. Scheme III depicts the formation of 2 different bridge groups, namely, the carbinol and methylene. All of these compounds are preferably formed from the (4-methoxyphenyl)-methanone base intermediate by the processes shown. Detailed descriptions of these processes, as with all other processes in Schemes I-II may be seen in the specific Examples later in this specification.

Finally, the preferred benzo[b]furan compounds can be synthesized via Friedel-Crafts acylation of the benzo[b]furan intermediate as outlined generally in Scheme IV below, and defined in detail in the specific Examples.

-12-

SCHEME IV

The general procedures for the above Scheme IV are referenced in J. Astoin, J. Heterocyclic Chemistry, 1977, Vol. 14, pp. 861-869.

- 5 The specific examples which follow are not to be considered as limiting the invention, but are merely illustrative of the best mode of synthesis of the most preferred compounds at this time.

-13-

Example 1Synthesis of α -(3'-Methoxyphenoxy)-4-methylacetophenone

51.05 g (0.24 mol) of 4-methyl- α -bromoacetophenone was dissolved in 200 mL of anhydrous (dimethyl formamide) DMF and 35g (0.28 mol) of 3-methoxyphenol was added. The reaction mixture was stirred with a mechanical stirrer and 80g (0.58 mol) of anhydrous K₂CO₃ was added. The reaction was allowed to proceed at ambient temperature and under an atmosphere of nitrogen. After eighteen hours, the reaction mixture was filtered to remove the inorganic salts. The DMF was removed by evaporation in vacuo, the resulting thick oil was dissolved in 250 mL of EtOAc and washed with brine. The EtOAc layer was removed and dried by filtration through anhydrous Na₂SO₄ and the solvent was removed by evaporation. The resulting oil was crystallized from Et₂O, which yielded 37g of the title compound as a light yellow solid.

PMR: consistent with the proposed structure

MS: m/e=256 (M+) FD

EA: Calc: C, 74.98; H, 6.29 Found: C, 75.17; H, 6.38

Example 2

Synthesis of 6-Methoxy-2-(4-methylphenyl)benzo[b]furan

30g (0.12 mol) of 2-(3-Methoxyphenoxy)-4'-methylacetophenone was dissolved in 300 mL of xylene and 45g of polyphosphoric acid (PPA) was added. The reaction mixture was stirred with a magnetic stirrer and kept under an atmosphere of nitrogen. The reaction was heated to reflux (135° C) for eighteen hours. The reaction was allowed to cool to about 90° C and 500 mL of water was added. The reaction was stirred until all the PPA dissolved in the water layer. The xylene layer was removed and extracted twice with water. The xylene layer was dried by filtration through

-14-

anhydrous Na_2SO_4 and evaporated to remove the solvent. The resulting solid was crystallized from hot ethyl acetate (EtOAc), which yielded 5g of the title compound as a white solid.

5

PMR: consistent with the proposed structure

MS: $m/e=238$ (M+) FD

EA: Calc: C, 80.65; H, 5.92 Found: C, 80.57; H, 5.97

$\text{C}_{16}\text{H}_{14}\text{O}_2$ MW= 238.29

10

Example 3

Synthesis of [6-Methoxy-2-(4-methylphenyl)benzo[b]furan-3-yl][4-cyanophenyl]methanone

15

One gram (4.2 mmol) of 6-Methoxy-2-(4-methylphenyl)benzo[b]furan was dissolved 25 mL of dichloromethane and 830 mg (5.0 mmol) of 4-cyanobenzoyl chloride was added. The reaction was stirred and kept under a nitrogen atmosphere. 2.75 mL (25 mmol) of TiCl_4 was slowly added to the reaction mixture. The reaction was allowed to proceed for four hours and quenched by the addition of 5 mL of methanol (MeOH). The solvents were removed by evaporation and the crude material was chromatographed on a silica gel column eluted with hexane-EtOAc (9:1). The desired fractions were determined by tlc and combined and evaporated to a solid. The resulting product was crystallized from diethyl ether (Et_2O). This yielded 325 mg of the title compound as yellow powder.

20

25

30

PMR: consistent with the proposed structure

MS: $m/e=368$ (M+) FD

EA: Calc: C, 78.46; H, 4.66; N, 3.81 Found: C, 78.28; H, 4.61; N, 3.52.

-15-

Example 4

Synthesis of [6-Methoxy-2-(4-methoxyphenyl)-benzo[b]
furan-3-yl]-3-(4-hydroxyphenyl)-methanone

5

10

15

20

A suspension of 2 g of [6-Methoxy-2-(4-methoxyphenyl)-benzo[b]furan-3-yl]-3-(4-methoxyphenyl)-methanone (5.15 mmol) and 1.3 g sodium ethanethiol (15.45 mmol) in 40 mL of DMF was heated to 80°C, checking by TLC 35% ethyl acetate:hexane every 20 minutes. When the starting material was consumed after 4 hours, the mixture was allowed to cool to room temperature, diluted with 150 mL. ethyl acetate, and washed twice with 1N H₂SO₄ (100 mL.). The organic layer was then dried on Na₂SO₄ and rotovaped to a brown oil, then purified by gravity chromatography with silica, 35% ethyl acetate:hexane. 1.15g yield (60%) of the title compound as a bright yellow solid. PChem: NMR QE300 MHz in d₆-DMSO: (3.85ppm, s, 3H), (3.90ppm, s, 3H), (6.85ppm, d, 2H), (6.95ppm, dd, 1H), (7.00ppm, d, 2H), (7.30ppm, ds, 1H), (7.40ppm, s, 1H), (7.60ppm, d, 2H), (7.75ppm, d, 2H).

Example 5

Synthesis of [6-Methoxy-2-(4-methoxyphenyl)-benzo[b]
furan-3-yl]-3-(phenyl-4-triflate)-methanone

25

30

35

A solution of 1.15 grams (3.07 mmol) of the Example 4 intermediate was dissolved in 40 mL. of methylene chloride with .92 mL. of tri-ethyl amine (6.61 mmol). To this was added a solution of 1.15 g of triflimide (3.23 mmol) in 10 mL. methylene chloride over 15 minutes. After addition, the mixture was allowed to stir at room temperature for 48 hours. The mixture was then diluted with 100 mL methylene chloride, washed twice with 1N H₂SO₄, twice with deionized water, twice with saturated NaHCO₃, then once more with water. Dry on Na₂SO₄ to yield 1.4 grams (89.7%) of the title compound yellow amorphous material. PChem: NMR QE300 MHz in CDCl₃: (3.80ppm, s, 3H), (3.90ppm, s, 3H), (6.75ppm, d, 2H),

-16-

(6.95ppm, dd, 1H), (7.10ppm, ds, 1H), (7.20ppm, d, 2H), (7.45ppm, d, 2H), (7.60ppm, d, 1H), (7.85ppm, d, 2H). MS FD+ = 506.

5

Example 6

Synthesis of [6-Methoxy-2-(4-methoxyphenyl)-benzo[b]
furan-3-yl]-3-(4-cyanophenyl)-methanone

A solution of 1.1 g of (2.17 mmol) of the Example 5
10 compound in 4 mL of dry acetonitrile with 156 mg KCN (2.39 mmol), 71 mg of nickel triphenylphosphine dichloride (.109 mmol), 57 mg of triphenylphosphine (.217 mmol), and 43 mg of zinc dust (.652 mmol) was heated to 65°C for 2 hours. The mixture was then allowed to cool to room temperature, diluted
15 with 100 mL of methylene chloride, washed twice with water, and dried on Na₂SO₄. The mixture was then purified on a silica plug with flash chromatography using 100% methylene chloride, rotovaped dry, and crystallized from 100% ethyl acetate to yield 580 mg of the title compound (70%). PChem:
20 NMR QE300 MHz in d₆-DMSO: (3.75ppm, s, 3H), (3.85ppm, s, 3H), (6.85ppm, d, 2H), (6.95ppm, dd, 1H), (7.35ppm, ds, 1H), (7.45ppm, d, 2H), (7.50ppm, d, 1H), (7.80ppm, s, 4H). MS FD+ = 384. EA calculated for C₂₄H₁₇NO₄ (Theory / Found): C, 75.19 / 75.35; H, 4.47 / 4.52; N, 3.65 / 3.66.

25

Example 7

Synthesis of [6-methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-
yl][4-methoxyphenyl]methanone

30 A solution of 1.54g (9 mmol) of 6-methoxy-2-(4-methoxyphenyl)benzo[b]thiophene and 1.62g (6mmol) of p-anisoyl chloride in 100 mL of methylene chloride was prepared and cooled to 0°C. Over 5 minutes, 1.20g (9mmol) of AlCl₃ was added in small portions. After 1 hour, the reaction
35 mixture was poured over 150 mL of ice water and extracted three times with 75 mL portions of methylene chloride. The methylene chloride extracts were combined and washed with 30

-17-

mL of 1 N NaOH, then with water. The organic layer was dried with magnesium sulfate and evaporated to dryness. The crude product was chromatographed on silica eluted with 30% EtOAc in hexane. The product was crystallized from acetone-methanol. This yielded 2.11g of the title compound as a light yellow solid.

PMR: consistent with the proposed structure

MS: m/e=404 (M+)

EA: Calc: C, 71.27; H, 4.98; S, 7.93; O, 15.82 Found C, 71.50; H, 5.00; S, 7.98; O, 15.77
C₂₄H₂₀O₄S

Example 8

Synthesis of [6-methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl][4-hydroxyphenyl]methanone

A solution of 0.4g (1 mmol) of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl][4-methoxyphenyl]methanone in 2 mL of DMF was added to a solution of 3 mL of 0.5 M sodium ethanethiol in DMF. The reaction mixture was heated to 80°C for 4 hours. The reaction mixture was allowed to cool and diluted with 10 mL of water and 10 mL of EtOAc and neutralized with 1 N HCl. The reaction mixture was extracted three times with 30 mL portions of EtOAc. The combined organic extracts were washed four times with 20 mL portions of brine and dried with MgSO₄ and evaporated to dryness. The crude product was chromatographed on silica eluting with 30 % EtOAc in hexane, and evaporated to dryness. This yielded 0.31g of the title compound as a yellow solid.

PMR: consistent with the proposed structure

MS: m/e=390 (M+) FD

EA: Calc: C, 70.75; H, 4.65 Found: C, 70.93; H, 4.56
C₂₃H₁₈O₄S

-18-

Example 9

5 Synthesis of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl][4-trifluoromethylsulfonyloxyphenyl]methanone

 A solution was prepared of 3g (7.7 mmol) of [6-methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl][4-hydroxyphenyl]methanone in 100 mL of methylene chloride and
10 1.57g (15.5 mmol) of triethylamine. A solution of 2.8g (7.72 mmol) of triflimide in 50 mL of methylene chloride was slowly added to the reaction mixture over a period of 30 minutes. The reaction, under an atmosphere of nitrogen and at room temperature, was allowed to proceed for 16 hours. The
15 reaction mixture was washed with 100 mL of 1 N H₂SO₄, then three times with 100 mL portions of 2N NaOH and finally with 100 mL of water. The organic layer was dried with anhydrous Na₂SO₄ and the product allowed to crystallize out. This yielded 2.5 g of the title compound as a yellow solid.

20

PMR: consistent with the proposed structure

MS: m/e=522 (M⁺) FD

EA: Calc: C, 55.17; H, 3.28 Found: C, 54.37; H, 3.21

25

Example 10

 Synthesis of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl][4-cyanophenyl]methanone

 A suspension of 2g (3.83 mmol) of the compound of
30 example 7 was prepared in 5 mL of anhydrous acetonitrile with 274 mg (4.21 mmol) of KCN, 125 mg (0.19 mmol) of nickel triphenylphosphine dichloride, 101 mg (0.38 mmol) of triphenylphosphine, and 38 mg (0.58 mmol) of zinc dust. The reaction mixture was heated to 65° C for 90 minutes under a
35 nitrogen atmosphere. The reaction mixture was allowed to cool to room temperature and was diluted with 100 mL of methylene chloride. The reaction mixture was filtered and washed twice

-19-

with 150 mL portions of water. The organic layer was dried with anhydrous Na_2SO_4 . The crude product was recrystallized twice from MeOH. This yielded 1.1g of the title compound as a yellow powder.

5

PMR: consistent with the proposed structure

MS: $m/e=399$ (M+) FD

EA: Calc: C, 72.16; H, 4.29; N, 3.51 Found: C, 71.96; H, 4.33; N, 3.50

10

$\text{C}_{24}\text{H}_{17}\text{NO}_3\text{S}$

Example 11

Alternate Synthesis of

[6-Methoxy-2-(4-methoxyphenyl)benzo[b]furan-3-yl][4-cyanophenyl]methanone

15

20

25

30

A solution of 160g (0.63 mol) of 6-methoxy-2-(4-methoxyphenyl)benzo[b]furan and 154.3g (0.93 mol) of 4-cyanobenzoyl chloride in 3L of methylene chloride was prepared. To this solution, 203.6 mL (1.85 mol) of TiCl_4 was slowly added. The reaction mixture was heated to reflux under a nitrogen atmosphere for a period of four hours. The reaction mixture was poured unto 3 kg of ice and allowed to quench for sixteen hours. The organic layer was separated and washed twice with 5 L portions of 20% NaHCO_3 solution and finally washed with 5 L of brine. The organic layer was dried with anhydrous MgSO_4 and evaporated to an oil. The crude product was chromatographed on a silica gel column eluted with 20% EtOAc- CH_2Cl_2 . This yielded 156g, which was further purified by rechromatography on a silica gel column eluted with 20% EtOAc- Et_2O and crystallized from the eluting solvent. This yielded 100.1 g of the title compound as a yellow powder.

-20-

Example 12

Synthesis of 6-Methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl] [4-n-propyloxyphenyl]methanone

5

A solution of 1.17g (3mmol) of [6-methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl] [4-hydroxyphenyl]methanone in 20 mL of DMF was prepared. To the solution was added 1.03g (7.50 mmol) of potassium carbonate and the mixture was heated to 100 °C. After 15 minutes, 2.73 mL (30 mmol) of 1-bromopropane was added and reaction was heated for an additional 40 minutes. The reaction mixture was allowed to cool and filtered. The filtrate was added to 25 mL of water and was extracted three time with 20 mL portions of EtOAc. The combined extracted were washed with brine, dried with MgSO₄ and evaporated to a solid. This yielded an eighty percent yield of the title compound.

PMR: consistent with the proposed structure
MS: m/e=432 (M⁺) FD
EA: Calc: C, 72.20; H, 5.59 Found: C, 72.00; H, 5.65
C₂₆H₂₄O₄S

Example 13

25 Synthesis of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl][4-n-butyloxyphenyl]methanone

In a manner identical with example 12, the title compound was prepared in 77% yield as a yellow solid.
30 PMR: consistent with the proposed structure
MS: m/e=446 (M⁺) FD
IR: 3011, 2963, 2938, 1599, 1476, 1254, 1166 cm⁻¹ (CHCl₃)

-21-

Example 14

Synthesis of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]furan-3-yl][4-methoxyphenyl]methanone

5

The title compound was prepared in a manner similar to that used in example 3, by using p-anisoyl chloride as the acylating agent.

10

Example 15

Synthesis of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]furan-3-yl][4-methoxyphenyl]methanol

15

20

25

A solution of 1g (2.56 mmol) of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]furan-3-yl][4-methoxyphenyl]methanone was prepared in 25 mL of THF. To this solution, 1g (25.4 mmol) of LiAlH₄ was added in small portions over a 10 minute period. The reaction was allowed to proceed at room temperature in a nitrogen atmosphere. After sixteen hours, the reaction was quenched by the addition of 5mL of water. To this suspension was added 3mL of 15%(w/w)NaOH and an additional 3mL of water. This suspension was filtered. The resulting filtrate separated into two layers. The top (organic) layer was removed and evaporated to dryness. The crude product was chromatographed on silica gel eluted with CHCl₃. This yielded 880 mg of the title compound as a colorless gum.

30

PMR: consistent with the proposed structure

MS: m/e=390 (M⁺) FD

EA: Calc: C, 73.83; H, 5.68 Found: C, 74.10; H, 5.66

-22-

Example 16

Synthesis of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]furan-3-yl][4-methoxyphenyl]methane

5 A solution of 400 mg (1.02 mmol) of [6-Methoxy-2-(4-methoxyphenyl)benzo[b]furan-3-yl][4-methoxyphenyl]methanol was dissolved in 10 mL of n-propylbenzene and 200 mg (5.25 mmol) of LiAlH₄ was added. The reaction mixture was heated to reflux under a nitrogen atmosphere for 45 minutes. After 10 sixteen hours, the reaction was quenched by the addition of 5 mL of water. To this suspension was added 3 mL of 15% (W/N) NaOH and an additional 3 mL of water. This suspension was filtered. The resulting filtrate separated into two 15 layers. The top (organic) layer was removed and evaporated to dryness. The crude product was chromatographed on silica eluted with 20% EtOAc in hexane. The final product was crystallized from ether. This yielded 100 mg of the title compound as a solid with a low melting point.

20 PMR: consistent with the proposed structure
MS: m/e=374 (M⁺) FD

25 Procedures for synthesizing other Formula I compounds are either well-known in the art or are easily converted by following one of the above procedures with different reagents, dependent upon the desired end compound.

The compounds of the present invention are preferably formulated prior to administration. Therefore, another 30 aspect of the present invention is a pharmaceutical formulation comprising a compound of formula I and a pharmaceutically-acceptable carrier, diluent, or excipient. The present pharmaceutical formulations are prepared by known procedures using well-known and readily available 35 ingredients. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a

-23-

carrier which may be in the form of a capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be a solid, semi-solid, or liquid material which acts as a vehicle, excipient, or medium for the active ingredient.

5 The compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, ointments containing for example up to 10% by weight of active compound, soft and hard gelatin capsules, suppositories, sterile injectable
10 solutions, and sterile packaged powders.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum, acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate,
15 microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propyl hydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving
20 agents, sweetening agents, or flavoring agents. Compositions of the inventions may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

25 The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 500 mg, more preferably about 25 to about 300 mg of the active ingredient. The most preferred unit dosage form contains about 10 to about 200 mg of the active ingredient. The term
30 "unit dosage form" refers to a physically discrete unit suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical
35 carrier. The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

-24-

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
active ingredient	250
Starch, dried	200
Magnesium stearate	<u>10</u>
Total	460 mg

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

A tablet is prepared using the ingredients below:

	Quantity (mg/tablet)
active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	<u>5</u>
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

-25-

Formulation 3

An aerosol solution is prepared containing the following components:

	Weight %
active ingredient	0.25
Ethanol	29.75
Propellant 22	<u>70.00</u>
(chlorodifluoromethane)	
Total	100.00

The active compound is mixed with ethanol and the mixture added to a portion of the Propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

Tablets each containing 60 mg of active ingredient are made as follows:

active ingredient	60 mg
Starch	45 mg
Microcrystalline cellulose	35 mg
Polyvinylpyrrolidone	4 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	<u>1 mg</u>
Total	150 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant

-26-

powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5

Capsules each containing 80 mg medicament are made as follows:

active ingredient	80 mg
Starch	59 mg
Microcrystalline cellulose	59 mg
Magnesium stearate	<u>2 mg</u>
Total	200 mg

The active ingredient, cellulose, starch and magnesium stearate are blended, passed through a No. 45 sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

Suppositories each containing 225 mg of active ingredient may be made as follows:

active ingredient	225 mg
Saturated fatty acid glycerides	<u>2,000 mg</u>
Total	2,225 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

-27-

Formulation 7

Suspensions each containing 50 mg of medicament per 5 ml dose
are made as follows:

	active ingredient	50 mg
10	Sodium carboxymethyl cellulose	50 mg
	Syrup	1.25 ml
	Benzoic acid solution	0.10 ml
	Flavor	q.v.
	Color	q.v.
15	Purified water to total	5 ml

The medicament is passed through a No. 45 mesh U.S.
sieve and mixed with the sodium carboxymethyl cellulose and
syrup to form a smooth paste. The benzoic acid solution,
flavor and color are diluted with some of the water and
added, with stirring. Sufficient water is then added to
produce the required volume.

Formulation 8

An intravenous formulation may be prepared as follows:

	active ingredient	100 mg
	Mannitol	100 mg
35	Purified water to total	200 ml

A variety of physiological functions have been shown to
be influenced by stimulation of the cannabinoid receptors.
The formula I compounds of the present invention are believed
to possess the ability to treat a variety of disorders in
mammals, including humans which are associated with
cannabinoid stimulation. Such disorders include, without
limitation, depression, cognitive dysfunction, loss of memory
and poor alertness and sensory perception. The formula I
compounds act as antagonists of the cannabinoid receptors.

-28-

Experiments were performed to demonstrate the antagonist activity of the formula I compounds at the cannabinoid receptors. The formula I compounds were tested for their ability to inhibit binding of $[1\alpha, 2\beta-(R)-5\alpha]-(-)-5-(1,1-$
5 $\text{dimethylheptyl})-2-[5\text{-hydroxy-2-(3-hydroxypropyl)cyclohexyl-phenol}(\text{CP-55,940})$; and for their ability to increase cAMP accumulation in cells.

10 **Cell Culture and Stable Expression of Cannabinoid Receptor Clones.**

Chinese hamster ovary cells (CHO) and murine Ltk cells were obtained from The American Type Culture Collection (Rockville, MD). CHO cells were maintained in an atmosphere of 5% CO₂ in growth media consisting of Alpha-MEM substituted
15 with 10% fetal calf serum, L-glutamine (2mM) and penicillin (50 U/ml) and streptomycin (50 ug/ml). Ltk cells were cultured at 37°C, in 5% CO₂ in Dulbecco's modified essential medium (0.45% glucose) containing fetal bovine serum (10%), L-glutamine (2 mM), penicillin (50 U/ml) and streptomycin (50
20 ug/ml). The rat cannabinoid receptor cDNA was stably expressed in CHO cells. A 2.2 kilobase (kb) SstI-EcoRI fragment containing the complete coding region of the human cannabinoid receptor gene was subcloned into the SstI and EcoRI sites of the pCD-PS vector to create plasmid hSKR6pl
25 which was transfected into CHO cells using calcium phosphate precipitation. Receptor-containing plasmids were co-transfected with plasmids containing the neomycin resistance gene created in a similar fashion. After transfection, the cells were selected with neomycin and the individual neomycin
30 resistant colonies grown to establish cell lines. The human cannabinoid (CB-1) cDNA was stably expressed in L cells with the following construct designed to amplify receptor expression levels thereby reducing the number of cells required for radioligand binding analysis. A 2.2 kilobase
35 (kb) SstI-EcoRI fragment containing the complete coding region of the human cannabinoid receptor gene was subcloned into the SstI and EcoRI sites of the pCD-PS vector to create

-29-

5 plasmid hSKR6pl. From hSKR6pl, a 3.3 kb Sall-NdeI fragment was removed, the ends blunted, and inserted into the blunted Sall site of the plasmid ptkmuARS-4. The resulting plasmid contained the receptor gene coding sequence flanked by the SV40 early region promoter and polyadenylation sequence originally engineered into the cloning vector pCD. This plasmid was transfected into murine Ltk cells by calcium phosphate precipitation. After transfection, L cells were selected in HAT medium. Individual HAT-resistant colonies were isolated after 3-4 weeks, grown to established cell lines, and cultured for at least 3 months to allow for expression of the receptor to stabilize.

10 Thus, two cell lines were established which expressed the human CB-1 receptor, i.e., the CHO and the Ltk.

15 Plasma Membrane Preparation

Ltk Cells, grown to confluency in 175 cm² culture flasks, were washed once with cold phosphate buffered saline and scraped in assay buffer (50 mM Tris, 5 mM EDTA, 5 mg/mL BSA, pH 7.4) with added 200 mM sucrose. Cells were then centrifuged at 1000 X g for 10 minutes at 4° C. The supernatant was discarded and the pellet resuspended in ice cold assay buffer, homogenized with a Tekmar Tissumizer (Cincinnati, OH, USA) at 95% maximal speed for 30 seconds followed by centrifugation for 15 minutes, 2-4° at 2000 X g. The supernatant was centrifuged again for 30 minutes at 43,000 X g. The pellet was resuspended in minimal volume of assay buffer containing 200 mM sucrose and stored at -80° until use.

30 All experiments were performed in glass test tubes, which were treated by soaking in dichloromethane/toluene (1:10 vol/vol) for 1 hour and then in methanol for 30 minutes, followed by a final rinse with 100% methanol. Test tubes were then allowed to air dry overnight before use.

-30-

Radioligand binding assays.

Competition and saturation binding assays were performed with [³H]CP55,940 as the labeled ligand. A rapid filtration binding assay was developed (see: Felder, C.C., et al., Proc. Natl. Acad. Sci., 90, p. 7656-7660(1993) based on a previously published method with the following modifications. All ligands were diluted in assay buffer containing 50 mg/mL fatty acid-free BSA, with the final BSA concentration not exceeding 5 mg/mL. Assay solutions were incubated in silicone-treated test tubes for 1 hr at 30°, with a final assay volume of 0.5 mL and a final membrane concentration of 40-400 µg of protein/mL. Membranes were rapidly filtered over GF/B filters (Whatman, Maidstone, England) that had been pretreated for 3 hr with 0.1% polyethyleneimine (v/w) (pH 7.4), using an Inotech (Lansing, MI) 96 or 48-position cell harvester. Membranes were washed with 3 x 3 mL of ice-cold wash buffer j (50 mM Tris, 0.5 mg/mL BSA, pH 7.4). Filters containing washed membranes were transferred to scintillation vials, 1 mL of 0.1% (v/v) Triton X-100 was added to each vial, and vials were incubated overnight before addition of scintillation cocktail (Hydrofluor; National Diagnostics, Manville, NJ). Protein concentrations were determined using the bioinchoninic acid protein reagent (Pierce, Rockford, IL), as described. Binding data were analyzed with the program LIGAND or with program GraphPad (GraphPad Software, San Diego, CA), which performs weighted nonlinear least squares curve-fitting to the general model of Feldman.

The results of the potent, specific binding of the compounds of formula I are demonstrated in Table 1.

-31-

TABLE 1

5	Compound	K _i versus [H ³] CP55,940
	Example 15	25800 nM
	Example 16	5300 nM
	Example 4	4120 nM
	Example 7	3600 nM
10	Example 12	1540 nM
	Example 8	950 nM
	Example 5	490 nM
	Example 13	430 nM
	Example 3	134 nM
15	Example 6	170 nM

Assay of cAMP accumulation.

The purpose of this assay is to demonstrate the effect of CB-1 binding compound on the CB-1 signal transduction pathway, i.e., to establish if the binding compound is working as an agonist or antagonist in vitro.

CHO cells were preincubated in growth media with forskolin (10⁻⁶ M) with or without anandamide for 4 hours. Cyclic AMP accumulation was measured over 5 minutes after exchanging the growth media with serum free media containing forskolin (10⁻⁶ M) and anandamide, with or without antagonists as indicated. Data are the mean \pm S.E. of three experiments performed in triplicate. The results for the compound of Example 6 are shown in Table 2. In this assay forskolin (FSK) raises the level of cAMP above the basal level (BSL). The addition of anandamide (ANM) inhibits the increase of cAMP induced by the forskolin. The compound of Example 6 inhibits the biological action of anandamide, i.e., negates the decrease in cAMP caused by anandamide, with an IC₅₀ of approximately 500nM.

-32-

TABLE 2

	Sample	cAMP Accumulation pM/mL
5	Baseline	<5
	Forskolin (FSK)	30
	Anandamide (ANM) (1 μ M)	<5
10	FSK + ANM	5
	FSK + Cmpd.6 (100 μ M)	35
	Cmpd.6 (100 μ M)	5
	FSK + ANM + Cmpd.6	
	Cmpd.6 (10nM)	5
15	Cmpd.6 (100nM)	10
	Cmpd.6 (1 μ M)	45
	Cmpd.6 (10 μ M)	65
	Cmpd.6 (100 μ M)	75

20 **Effect on N-type Calcium Channel Currents**

25 In this series of experiments, it is demonstrated that compounds of formula I, not only bind the CB-1 receptor and inhibit the signal transduction pathway of CB-1, when activated by its endogenous ligand, anandamide, but in addition, effect other nerve cell organelles under control of the CB-1 signaling pathway *in vitro*. Specifically, the compounds of formula I open the N-type calcium channels, which are closed by either anandamide or the cannabinoids (see: Mackie, K. and Hille, B., Proc. Natl. Acad. Sci., **89**, p.3825-3829 (1992)).

30 **Materials.**

35 DMEM was obtained from Biowhittaker and GIBCO, FBS from HyClone, bovine serum albumin (fatty-acid free), dimethylsulfoxide, and NEM from Sigma, PTX from List, ω -CgTX from Peninsula Labs, and tetrodotoxin from Calbiochem. WIN 55,212-2 was a gift from Sterling Research Group. CP 55,940

-33-

was a gift from Pfizer Central Research. Anandamide was synthesized as described previously. Purity was monitored using thin layer chromatography with an elution system of petroleum ether/ether/methanol (in a ratio of 6:40:4).
5 Anandamide migrated as a single spot with R_F of 0.5, as expected.¹

Cell culture and preparation.

10 N18 (neuroblastoma cell line) cells (passages 32-41) were grown on glass coverslip fragments in DMEM plus 5% (fetal bovine serum) (FBS, using standard cell culture techniques. Six to 14 days before recording, cells were "differentiated" by changing the medium to DMEM plus 0.5% FBS plus 2% dimethylsulfoxide. In PTX (Pertussis Toxin)
15 experiments, differentiated cells were grown for an additional 16-20 hours in medium containing 500 ng/ml PTX. Control cells were treated identically, except that neurons were provided by M. S. Shapiro (University of Washington, Seattle, WA).

Current recording.

20 Currents were recorded using the whole-cell voltage-clamp technique. Pipettes were pulled from hematocrit glass (VWR) and fire polished. The pipette solution contained (in
25 mM) 100 CsCl, 10 EGTA, 5 MgCl₂, 40 HEPES, 3 Na₂ATP, and 0.2 GTP, pH 7.30 with CsOH. For recording, a coverslip containing cells was transferred to the recording chamber (200 μ l) and constantly perfused at a rate of 1-2 ml/min with an external solution containing (in mM) 160 NaCl, 5 CaCl₂, 4
30 KCl, 1 MgCl₂, 10 HEPES, and 8 glucose, pH 7.35 with NaOH. Tetrodotoxin (200 nM) was added to block voltage-gated sodium currents, and bovine serum albumin (3 μ M) was present in all recording solutions to decrease adsorption of cannabinoids. Subsequently the cannabinoid agonist WIN 55,212 and the
35 compounds of this invention were added. I_{Ca} was measured near the end of a 25-msec depolarizing pulse to 0 mV and was defined as that component of the current sensitive to 100 μ M

-34-

CdCl₂. Solution reservoirs were selected by means of a series of solenoid valves, and solution changes were accomplished in <1 min. In all experiments the cells were held under voltage clamp at a holding potential of -65 mV. Voltage protocols were generated and data were digitized, recorded, and analyzed using BASIC-FASTLAB (Indec Systems, Capitola, CA). Currents were sampled at 4kHz and junction potentials are uncorrected. To control for potential response variations with passage number and duration of differentiation, experimental and control measurements were alternated whenever possible. Where appropriate, data are expressed as mean \pm standard error. [It has been shown in this type of experiment that WIN 55,212 (used for convenience) acts the same as anandamide, see: Felder, et al; ibid.]

The data from this experiment is shown in Table 3 for the compound of Example 6. The agonist WIN 55,212 inhibits the N-type calcium channel via the CB-1 receptor, thus decreasing the current to the voltage clamp of -65 pA. The addition of a compound of formula I (Example 6) increases this current to -150 pA by opening the N-type calcium channel via the CB-1 receptor. Oxo-M and Cd²⁺ are controls showing the specificity of the current regulation and the integrity of the cellular organelles.

Thus, this experiment demonstrates the ability of the compounds of formula I to regulate major nerve cell organelles by antagonizing the actions of either the endogenous ligand, anandamide or agonists *in vitro*.

-35-

TABLE 3

Sample	Cell Current pA
Baseline	-150
WIN 55,212 (WIN) (100nM)	-60
Cmpd. 6 (1 μ M)	-150
WIN+Cmpd. 6	-150
Oxo-M+Cmpd. 6	-60
Cd ⁺⁺	-60

In Vivo Effects of Anandamide Antagonists

The following experiment demonstrates the ability of the compounds of formula I to inhibit the sedation caused by anandamide *in vivo* and in a standard model (Open Field Assay) of mouse behavior, which has been a classic model for the evaluation of active and useful central nervous system agents.

The Open Field Assay evaluates the motion (both horizontal and vertical) of a mouse when placed in a large, open area. Specifically, C57BL/6J mice are injected with 20mg/kg (i.p.) of the compound of Example 6, 28 minutes before injecting anandamide at 2 mg/kg (i.p.). The mouse is placed in the center of a large area of a device which measures the number of vertical and horizontal movements of the mouse (Digiscan Automated Open Field). Each test session is of 5 minute duration. At the end of this time period, the mouse is removed from the scanner and the recorded data are automatically analyzed and statistically evaluated. Further details are given in Crawley, J., J. Neurosci., 12(9), p. 3380-3391 (1992).

The results of this experiment are shown in Tables 4-5. As seen in the Tables, anandamide causes a severe depression and sedation of the animal's movements, which is reversed by the administration of the compound of Example 6 in regard to

-36-

Horizontal Exploratory Activity and Total Distance
Exploratory Behavior.TABLE 4

5 Total Distance Exploratory Behavior

	Sample	Digiscan Total Distance/5min.
10	Vehicle	250*
	Vehicle+Anandamide	50
	Cmpd.6 +Anandamide	300*

*p<0.01

15

TABLE 5

Horizontal Exploratory Behavior

20	Sample	Digiscan Horizontal Activity/5min.
	Vehicle	1100*
	Vehicle+Anandamide	200
25	Cmpd.6 +Anandamide	900*

*p<.05

Applications

30 The compounds of formula I have been shown to bind to
the human CB-1 receptor and to inhibit the cellular, signal-
transduction events evoked by both anandamide and
cannabinoids. *In vivo*, the compounds have been shown to
mitigate the effects of anandamide in the mouse. Due to the
35 localization of the CB-1 receptor in the hippocampus and the
known pharmacology of the cannabinoids and their
correspondence with anandamide (see: Howlett, A.C., et al.,

-37-

TINS, 13(10),p. 420-423.(1990)), mammals, including humans, suffering from symptomology similar to that seen with cannibinoids, would recieve benefit from an antagonist of the CB-1 receptor (a compound of formula I). Many disease states, although caused by different etiologies, have common symptomology. The disease states (listed in the applications, below) are not caused by the use or abuse of cannibinoids, but since their symptoms are so similar, the endogenous factor anandamide would seem to be a likely contributory factor. Thus, a compound of formula I would be of benefit in the treatment of the symptomology of many diseases. Additionally, since treatment of symptomology and treatment of the causal factors of a disease and their interaction are not well understood or, indeed, may not be separate pathologies, it should not be construed that this invention would be solely limited to the treatment of symptoms.

Symptoms, which would be benecially effected in mammals, including humans, with a compound of formula I, would include, but not be limited to: depression, loss of cognitive function, loss of mental alertness, loss of memory, and loss of sensory perception. These symptoms occur in a variety of pathological states, syndromes, and diseases. The extent, magnitude, particulars of these symptoms vary widely both between disease states and between various individuals suffering from one of those diseases.

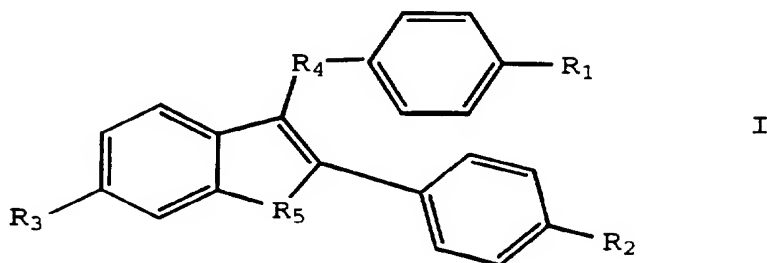
Listed, below, are some of the diseases, from which mammal, including a human, would derive a benefit from a compound of formula I: Alzheimer's Disease, head trauma, senile dementia, brain tumors, and the like. Additional, disease states which may be included in this catagory are elaborated in voluminous references in the art, e.g., see: "Harrison's Priciples of Internal Medicine" , Isselbacher, K.J., et all. Eds. 9th Ed., McGraw-Hill Book Co., New York,1980, Section 3, "Alterations in Nervous Function". Again, due to variability of symptoms in any of these diseases, the decision of the use, dosage level, and

-38-

protocol of treatment of a compound of this of this invention
is at the discretion of the attending physician.

We Claim:

1. Use of a compound of formula



in which:

R^1 is C₁-C₄ alkoxy, trifluoromethylsulfonyloxy, hydroxy or cyano;

R^2 and R^3 are each independently C₁-C₄ alkyl or C₁-C₄ alkoxy;

R^4 is CO, CHOH or CH₂; and

R^5 is O or S, provided that when R^1 is hydroxy, R^5 is O, for the manufacture of a medicament for use as an antagonist of anandamide at Cannabinol-1 receptors.

2. Use as claimed in Claim 1, in which R^2 is methyl or methoxy and R^3 is methoxy.

3. Use as claimed in Claim 2, in which R^1 is methoxy, trifluoromethylsulfonyloxy, hydroxy or cyano.

4. Use as claimed in Claim 3, in which R^1 is cyano.

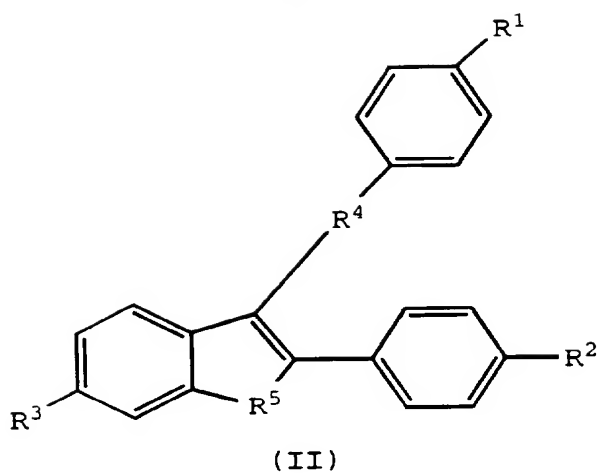
5. Use as claimed in Claim 4, in which R^2 is methoxy and R^5 is -O-.

6. Use as claimed in Claim 4, in which R^2 is methyl and R^5 is -O-.

7. Use as claimed in Claim 4, in which R^2 is methoxy and R^5 is -S-.

8. A compound of the formula:

40



Wherein

R¹ is cyano;

R² and R³ are each individually, C₁-C₄ alkyl or C₁-C₄ alkoxy;

R⁴ is carbonyl; and

R⁵ is oxygen or sulfur.

9. The compound of Claim 8 wherein R² and R³ are both methoxy and R⁵ is -O-.

10. The compound of Claim 8 wherein R² is methyl, R³ is methoxy and R⁵ -O-.

11. The compound of Claim 8 wherein R² and R³ are both methoxy and R⁵ is -S-.

12. A pharmaceutical formulation comprising an effective amount of a compound of Claim 8, and a pharmaceutically acceptable carrier, excipient or diluent thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08455

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/38, 31/34; C07D 333/54, 333/56, 307/79, 307/80

US CL : 549/49, 57, 58, 462, 468, 469; 514/443, 469

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 549/49, 57, 58, 462, 468, 469; 514/443, 469

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE SEARCH: STRUCTURE SEARCH, APS SERCH: Search terms: anandamide, cannabinoid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INDIAN JOURNAL OF CHEMISTRY, Vol 22B, issued May 1983, Durani et.al, " A Convenient Synthesis of 2-Aryl-3-aryloxybenzo[b]furans", pages 489-490, see page 489.	8-10, 12
Y	US, A, 5,308,866 (JONES ET.AL.) 09 January 1994, see column 1 and 2.	1-12
Y	US, A, 5,308,866 (LESIEUR ET.AL.) 03 May 1994, column 1 and 2.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

27 SEPTEMBER 1995

Date of mailing of the international search report

19 OCT 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

SHANLENDRA KUMAR

Facsimile No. (703) 305-3230

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08455

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☒

The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08455

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-6, 8-10 and 12, drawn to compounds when R5 is oxygen.

Group II, claims 1-4, 7-8 and 11-12, drawn to compounds when R5 is sulfur.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Compounds of Group I are benzofurans as against benzothiophenes of Group II. These two structures are chemically disparate (no common core) and there is no common technical feature associated with them. A reference anticipating claims of Group I may not be a reference to the claims of Group II.